

could be obtained within 30 min using only 20 µL serum or tryptase calibrators and showed excellent conformity to the commercial tryptase assay.

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# **An inhibition ELISA for the determination of major allergen group 1 in different grass species**

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**Background:** Allergy induced by grass pollen can be reduced by means of sublingual allergen-specific immunotherapy (IT). One of the major allergens in grass pollen is grass Group 1. Two antibody-based methods, a sandwich-ELISA and an inhibition ELISA, were developed for the quantification of grass Group 1 in grass pollen extracts. Both methods were compared in order to determine the most appropriate assay for use of quantification of Group 1 in a grass pollen IT product that consists of four different grass pollen extracts.

**Method:** The sandwich ELISA uses two specific monoclonal antibodies (7E7 and 1B8) against Lol p1 major allergen, one on the solid phase and one as detecting antibody. The inhibition ELISA (iELISA) uses one specific monoclonal antibody (7E7) that is mixed with grass pollen extract and recombinant Phleum p1 allergen on the solid phase. The products tested were pollen extracts of four different grass species, i.e. *Phleum pratense*, *Lolium perenne*, *Poa pratensis* and *Secale cereale*. The standards used are well characterised grass Group 1 allergens (Phl p1, Lol p1, Poa p1 and Sec c1) purified from grass pollen extracts.

**Results:** The sandwich ELISA showed different curves (e.g. non-parallel lines, different OD maxima) when grass pollen extracts from different grass species were analysed, while the iELISA showed similar curves for all four grass species. Further development of the sandwich-ELISA would involve separate development of 4 methods with different conditions, while only one method could be used with the iELISA for these extracts of 4 grass species. Therefore, the iELISA was chosen for further development and qualification. Qualification showed that the iELISA generates reproducible results with low variation.

**Conclusion:** Quantification of grass Group 1 major allergen in four different grass species can be done with one method using an iELISA. The sandwich ELISA was less suitable since different test conditions were

needed when testing different grass species. The newly developed iELISA can precisely quantify Group 1 allergen in pollen extracts from *Phleum pratense*, *Lolium perenne*, *Poa pratensis* and *Secale cereale*.

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# **Sensitisation to Act d 2 in patients allergic to *Alternaria alternata*: an epiphenomenon without clinical significance?**

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**Background:** In the last few years, the introduction of microarrays in the diagnosis of type I allergy is allowing the clinicians to have a much more accurate picture of their allergenic profile. However, the simultaneous measurement of specific IgE to multiple molecules can show unexpected sensitisations, without knowing their clinical relevance. For instance, we have been observing a high prevalence (74%) of sensitisation to Act d 2 (the thaumatin of kiwifruit) in patients sensitised to Alt a 1 (major allergen of *Alternaria alternata*) with a confirmed allergy to this mould. The aim of the present study was to clarify if there was any clinical relevance in this finding.

**Method:** We selected patients allergic to *A. alternata* (rhinitis and/or asthma) with a positive specific IgE (sIgE) to Alt a 1 and Act d 2 in the allergen microarray immunoassay (ImmunoCAP ISAC, ThermoFisher Scientific).

Skin prick tests (SPT) were performed with the commercial available *A. alternata* and kiwifruit extracts (Laboratorios LETI), a purified nAct d 2 protein and a prick by prick with fresh kiwifruit. Total IgE and sIgE (UniCAP, ThermoFisher Scientific) to kiwifruit and *A. alternata* were obtained, and an open oral food challenge (OFC) with kiwifruit was also carried out.

**Results:** Eighteen patients were selected, nine of them were skin prick tested and orally challenged. 9 men, 9 women, median total IgE 195 kU/l (interquartile range (IQR) 128–542). sIgE to kiwi was negative in all cases, median sIgE to *A. alternata* was 9.86 kU/l (IQR 5.5–16.8), median sIgE to Act d 2 was 1.9 ISU (0.9–4.6), and sIgE to Alt a 1 was 9.8 ISU (2.5–20.4). A correlation was found between sIgE to Act d 2 and sIgE to Alt a 1 (Rho Spearman = 0.686; significance = 0.01).

SPT to *A. alternata* extract was positive in all cases, SPT to commercial kiwi, prick by prick with fresh fruit and nAct d 2 were all negative. OFC with kiwifruit was well tolerated in all patients.

**Conclusion:** In our population, sensitisation to Act d 2 in the context of an allergy to *A. alternata* has no clinical relevance. This sensitisation could only be seen with the microarray immunoassay, SPTs and sIgE to kiwi failed to detect it.

A significant correlation was found between sIgE to Act d 2 and Alt a 1. Further studies are needed to confirm if this sensitisation is real and in second place, to figure out if there could be any other link between both allergens rather than the clinical one.

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# **Stability of purified nsLTP prick tests by *in vitro* analysis**

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**Background:** The use of purified allergens as a diagnostic for skin prick test allows the identification of real sensitisation to a specific allergen present in a protein extract and it is a very useful tool for the study of cross-reactivity. However, the quality of these products has to be demonstrated. In that sense, there is no studies about the stability of purified proteins as reagents for diagnosis. The objective of the study was to determine the stability of purified nsLTP prick tests under different storage conditions.

**Method:** Pru p 3 and Cor a 8 were purified in an ÄKTAexplorer FPLC and used to prepare prick tests with a concentration of 30 µg/ml of total LTP. A mixture of purified LTPs (15 µg/ml of Pru p 3 and 15 µg/ml of Cor a 8) was selected in order to increase the diagnosis efficacy. After formulation, pricks were stored at three temperatures (room temperature, 4° and –20°C) and they were analysed monthly by SDS-PAGE and immunoblot. Pru p 3 or Cor a 8 polyclonal rabbit antibodies were used in immunoblot experiments. Finally, pH and glycerol valuation was also carried out.

**Results:** SDS-PAGE and immunoblot results showed that there were not important differences in protein molecular weights or protein band intensity between the 3 temperatures and during the whole period of study (6 months). Immunoblot results showed a slight decrease in the intensity of Cor a 8 recognition after 6 months. Regarding pH and glycerol values, both parameters were maintained in the range of 7.5–8.5 and 45–55%, respec-